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Syndrome of the month

Alagille syndrome

Ian D Krantz, David A Piccoli, Nancy B Spinner

Abstract

Alagille syndrome (OMIM 118450) is an autosomal dominant disorder associated with abnormalities of the liver, heart, eye, skeleton, and a characteristic facial appearance. Also referred to as the Alagille-Watson syndrome, syndromic bile duct paucity, and arteriohepatic dysplasia, it is a significant cause of neonatal jaundice and cholestasis in older children. In the fully expressed syndrome, affected subjects have intrahepatic bile duct paucity and cholestasis, in conjunction with cardiac malformations (most frequently peripheral pulmonary stenosis), ophthalmological abnormalities (typically of the anterior chamber with posterior embryotoxon being the most common), skeletal anomalies (most commonly butterfly vertebrae), and characteristic facial appearance. Inheritance is autosomal dominant, but expressivity is highly variable. Sibs and parents of probands are often found to have mild expression of the presumptive disease gene, with abnormalities of only one or two systems. The frequency of new mutations appears relatively high, estimated at between 15 and 50%. The disease gene has been mapped to chromosome 20 band p12 based on multiple patients described with cytogenetic or molecular rearrangements of this region. However, the frequency of detectable deletions of 20p12 is low (less than 7%). Progress has been made in the molecular definition of an Alagille syndrome critical region within the short arm of chromosome 20. We will review the clinical, genetic, cytogenetic, and molecular findings in this syndrome.

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The association of cholestasis with pulmonary artery hypoplasia or stenosis, minor skeletal features, and unusual facial appearance was initially described by Alagille *et al* in 1969[1] and by Watson and Miller in 1973.[2] Subsequent reports described characteristic ocular abnormalities.[3] Alagille syndrome (syndromic bile duct paucity or arteriohepatic dysplasia) is characterised by reduction in the number of bile ducts on biopsy leading to the obstruction of biliary flow (cholestasis), in association with cardiac, skeletal, ocular, facial, and, less frequently, renal and neurodevelopmental abnormalities.[4] The prevalence of this syndrome has been reported as 1 in 100 000 live births when probands were ascertained based on the finding of neonatal liver disease.[5] The familial nature of the disorder has been recognised from early reports[6 7] and subsequent studies have shown autosomal dominant inheritance, with highly variable expression. Cytogenetic abnormalities in multiple Alagille syndrome patients have mapped the disease gene to 20p12.

Clinical features

HEPATIC MANIFESTATIONS

The majority of symptomatic patients present in infancy with manifestations of hepatic disease ranging from mild cholestasis and pruritus to progressive liver failure. The extremely variable expression of hepatic disease even occurs within families and, based on studies of parents and sibs of affected children, it appears that some gene carriers may not have any detectable hepatic manifestations.[6 7] Jaundice is present in the majority of symptomatic patients and presents as a conjugated hyperbilirubinaemia in the neonatal period. Cholestasis is manifest by pruritus, raised serum bile acid concentrations, xanthomas, and growth failure. The pruritus associated with Alagille syndrome is among the worst of any chronic liver disease. It is rarely manifest before 3 months of age, but is seen in most affected children by the third year of life, even in some who are anicteric.[8] There is currently no way to predict which patients with neonatal liver disease will progress to end stage liver disease and require transplantation. Progression to cirrhosis and liver failure occurs in a significant proportion of patients, with approximately 15% requiring transplantation (accounting for 2% of all paediatric liver transplants).[9] Growth failure has been reported in 50-90% of patients,[4 10] most likely as a result of malnutrition, caused by poor solubilisation and absorption of dietary lipids, essential fatty acids, and fat soluble vitamins.

Laboratory findings most commonly include raised serum bile acids, conjugated bilirubin,

Table 1 Frequency of major clinical criteria in Alagille syndrome patients

Study	*M/F*	*Liver*	*Heart*	*Eye*	*Vertebral*	*Facies*	*Other*
4	?	73/80 (91%)	68/80 (85%)	55/62 (88%)	70/80 (87%)	76/80 (95%)	Renal 17/23 (74%) MR (16%)
10	16/11	25/27 (93%)	26/27 (96%)	9/16 (56%)	6/18 (33%)	19/27 (70%)	
13	3/4	7/7 (100%)	7/7 (100%)	2/3 (67%)	4/5 (80%)	3/7 (47%)	MR 2/3 (67%)
7	4/2	6/6 (100%)	6/6 (100%)	?	5/6 (83%)	6/6 (100%)	MR 1/6 (17%)
46	5/1	6/6 (100%)	6/6 (100%)	0/6* (0%)	1/6† (17%)	6/6 (100%)	Clinodactyly 4/6 (67%)
3	4/1	5/5 (100%)	4/5‡ (80%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	Short dist phal 4/5 (80%) Short ulnae 3/5 (60%) Renal 2/5 (40%) Hypothyroid 1/5 (20%)
47	2/3	5/5 (100%)	5/5 (100%)	?	3/5 (60%)	5/5 (100%)	Prox thumbs 2/5 (40%)
21	0/3	3/3 (100%)	3/3 (100%)	1/3 (33%)	3/3 (100%)	3/3 (100%)	JRA 1/3 (33%)
Our patients	28/23	56/56 (100%)	44/44 (100%)	34/41 (83%)	29/53 (59%)	40/40 (100%)	Renal 13/56 (23%) Panc insuff 2/56 (4%)
Total	62/48	95%	92%	78%	70%	91%	Renal 38%

M = male, F = female, JRA = juvenile rheumatoid arthritis.
* Two patients with ocular findings other than posterior embryotoxon.
† Five patients with decreased interpeduncular distance.
‡ One patient with isolated VSD.

alkaline phosphatase, cholesterol, and gamma-glutamyl transpeptidase, indicative of a defect in biliary excretion. Less frequently, raised serum aminotransferases and triglycerides may be present. Hypercholesterolaemia and triglyceridaemia may be profound in severe cholestasis. Liver biopsy classically shows intrahepatic bile duct paucity, although the diagnostic histopathological lesion of intralobular bile duct paucity is progressive and may not be evident in the newborn period. Depending on when a biopsy is performed, there may be a broad range of histological findings including portal fibrosis and, rarely, bile duct proliferation.[11]

CARDIAC MANIFESTATIONS

Congenital heart disease has been reported in up to 90% of patients with Alagille syndrome. The most common form of heart defect involves the pulmonary valve, artery, and branches with the most common finding being peripheral pulmonary stenosis. The most common complex cardiac malformation associated with pulmonary involvement in these patients is tetralogy of Fallot (7-10%).[4 10] Other cardiac defects seen in association with Alagille syndrome, listed in order of decreasing frequency, include ventricular septal defects, atrial septal defects, aortic stenosis, and coarctation of the aorta.[12] While the majority of cardiovascular malformations are haemodynamically insignificant, the more severe malformations have accounted for the majority of early mortality in some series.[10 13]

OPHTHALMOLOGICAL MANIFESTATIONS

Ophthalmological findings in patients with Alagille syndrome predominantly include defects of the anterior chamber (posterior embryotoxon, Axenfeld's anomaly, or Rieger anomaly), and retinal pigmentary changes.[4 10 14] Posterior embryotoxon (a prominent centrally positioned Schwalbe's ring at the point of joining of the corneal endothelium and uveal trabecular meshwork) (fig 1) has been reported in up to 89% of patients and is therefore very important diagnostically. Posterior embryotoxon also occurs in the general population with a frequency of 8-15%,[15] which at times provides for diagnostic dilemmas in otherwise unaffected family members of Alagille syndrome probands. A spectrum of retinal pigmentary changes have been reported in Alagille syndrome patients and although initially assumed to be because of dietary deficiency, these changes are seen in patients with normal levels of vitamin A and E.[4 10] In the majority of patients, visual prognosis is good, although mild decreases in acuity have been reported.[14 16-18] Other, less frequently reported ophthalmological findings include microcornea, keratoconus, congenital macular dystrophy, shallow anterior chambers, exotropia, ectopic pupil, band keratopathy, choroidal folds, and anomalous optic discs.[14 16-18] The finding of ocular abnormalities other than posterior embryotoxon (which has a relatively high prevalence in the general population) can aid in establishing a diagnosis.

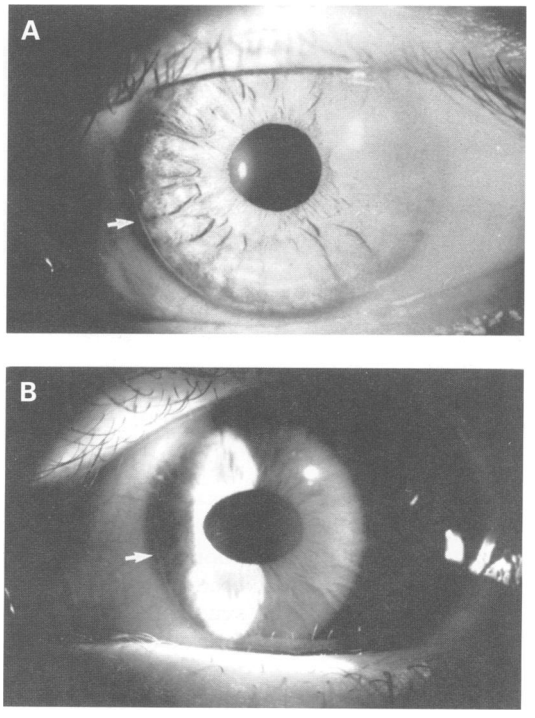

Figure 1 Posterior embryotoxon seen in a father and daughter with Alagille syndrome. Arrow points to the prominent Schwalbe's line in (A) father (anterior segment photograph) and (B) the daughter (slit lamp photograph). (Photographs courtesy of Dr Stephen Orlin, Scheie Eye Institute, Philadelphia, PA, USA.)

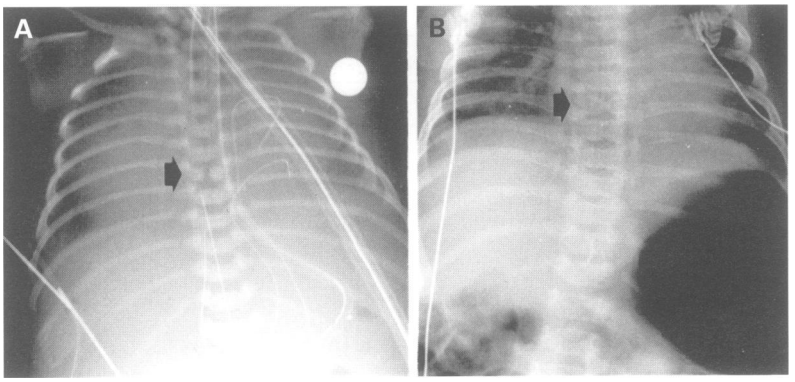

Figure 2 Butterfly vertebrae. Arrows point to butterfly vertebrae identified in two patients with Alagille syndrome. Note more severe clefting of vertebrae in patient on the left.

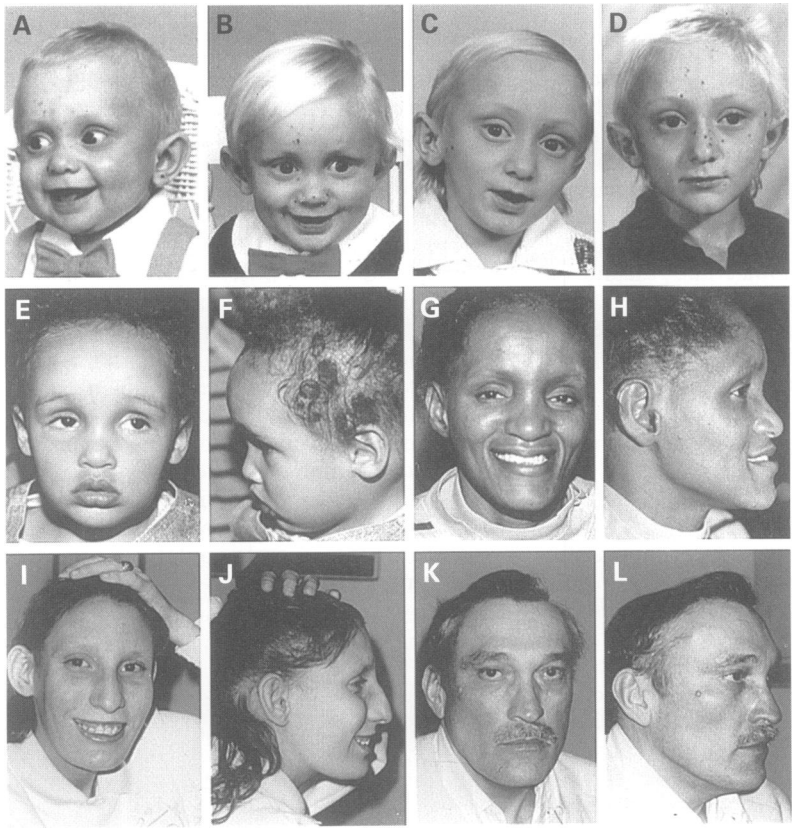

Figure 3 Characteristic facial features of Alagille syndrome. Photos (A-D) show the same patient at 1 year, 2 years, 4 years, and 6 years, respectively. Note evolution of features with loss of baby fat as well as excoriations secondary to severe pruritus as a result of cholestasis (reproduced with parental consent). Photos (E-H) show full face and profile views of an affected mother and daughter, while photos (I-L) depict a father and daughter with Alagille syndrome (photos (I-L) from Spinner et al, Am J Hum Genet, 1994, reproduced with permission from the University of Chicago Press).

SKELETAL MANIFESTATIONS

The most common skeletal abnormality in Alagille syndrome is butterfly vertebrae. Butterfly vertebrae result from clefting abnormalities of the vertebral bodies resulting in a "flying butterfly" appearance on radiological examination (fig 2). The frequency of butterfly vertebrae is 70% in reported cases of Alagille syndrome (table 1). We have studied a series of 53 patients, and identified butterfly vertebrae in 59%.[19] The incidence of butterfly vertebrae in the general population is unknown as they are for the most part asymptomatic and are usually found incidentally. Other reported skeletal anomalies include narrowing of interpeduncular spaces in the lumbar spine (50%), pointed anterior process of C1, spina bifida occulta, fusion of adjacent vertebrae, hemivertebrae, bony connections between ribs, and short fingers.[2 4 10 20 21]

CHARACTERISTIC FACIES

The constellation of facial features seen in Alagille patients include a prominent forehead, deep set eyes with moderate hypertelorism, pointed chin, and saddle or straight nose with bulbous tip. The combination of these features give the face a triangular appearance (fig 3). Detractors argue that there is inter-observer variability in identification of these features and that they are a common result of early and chronic cholestasis ("cholestasis facies") rather than typical of Alagille syndrome.[22]

Other features

Renal anomalies have been reported in from 23 to 74% of patients in those studies that examined renal function (table 1). Renal defects include functional and structural abnormalities.[2 4 23 24] In Alagille's review of 80 cases,[4] he noted delayed puberty and high pitched voice, but these have not been consistently reported. Hearing loss was described in a large kindred reported by LaBrecque *et al.*[23] While this has not been reported in any of the large patient studies, hearing loss has been reported in patients with Alagille syndrome and cytogenetically visible deletions of 20p.[25] Neurovascular accidents have been reported in Alagille syndrome with occurrences as high as 15%.[26]

COGNITIVE FUNCTIONING

In the earlier reports of this syndrome Alagille noted significant mental retardation (IQ 60 to 80) in nine of 30 patients studied.[27] Later studies did not find such a high frequency of mental retardation, although evidence of delayed development was observed in some patients.[4 10] This is most likely secondary to better recognition of the disease early on with more aggressive nutritional management and intervention.

PROGNOSIS

The outcome and prognosis is highly variable and is directly related to the severity of the liver or cardiac involvement or both with mortality equally attributable to both of these organs. Complex congenital heart disease is responsible for most of the neonatal deaths, while liver failure accounts for most of the later morbidity and mortality.

Differential diagnosis

Diagnosis of Alagille syndrome can be difficult. There are over a hundred specific causes of neonatal cholestasis, and there are no clinical, biochemical, radiological, or histological findings specific for Alagille syndrome. Emergent and treatable causes of neonatal cholestasis (such as sepsis and galactosaemia) should first be considered. Cholestasis resulting from extrahepatic causes such as biliary atresia can be differentiated by a DISIDA scan. Extrahepatic structural duct abnormalities such as choledochal cysts can be eliminated from consideration

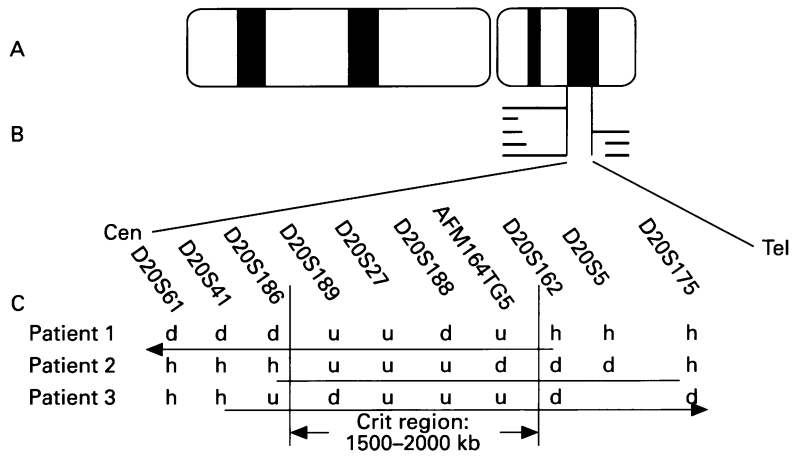

Figure 4 Alagille syndrome critical region within chromosome 20p12. (A) Ideogram of chromosome 20. (B) Published representative deletions of Alagille patients showing overlap within 20p12. (C) Enlarged view of 20p12 showing microsatellite markers spanning the critical region and analysis in three patients.[19] "d" indicates deletion of that region, "h" indicates the patient was heterozygous (not deleted) at this locus, and "u" indicates that the patient was uninformative.

by hepatic ultrasound. A liver biopsy will usually show bile duct paucity in Alagille syndrome, although a picture suggestive of neonatal hepatitis or even bile duct proliferation can be seen.[11] Even bile duct paucity is not specific for Alagille syndrome (and, we hypothesise, not even necessary for the diagnosis). The specific diagnosis of the Alagille syndrome is based solely on the clinical phenotype. In the study by Alagille *et al*,[4] 73/80 patients had cholestasis. All of those with cholestasis had at least two other major features. Alagille *et al*[4] suggested that the diagnosis can be made based upon the presence of cholestasis and two other major criteria, while in those patients without cholestasis the diagnosis may be more difficult. The relatively common occurrence of transient jaundice (indirect hyperbilirubinaemia), and peripheral pulmonary stenosis in neonates as well as the high population incidence of posterior embryotoxon (8-15%), and the subjective assessment of the facies, can present historical and diagnostic dilemmas and heralds the need for more specific diagnostic criteria.

Other disorders associated with intrahepatic cholestasis, although rare and in many cases limited to specific populations, include autosomal recessive Byler syndrome, autosomal recessive Norwegian cholestasis (Aegenaes syndrome), North American Indian cholestasis (NAIC), autosomal recessive benign recurrent intrahepatic cholestasis (BRIC), Zellweger syndrome, and α-1-antitrypsin deficiency.

Posterior embryotoxon is seen in Rieger syndrome, Bannayan-Riley-Ruvalcaba syndrome, and numerous other anterior chamber cleavage syndromes, as well as in 8-15% of the normal population. Stenosis at various points in the pulmonary tree is seen both in isolation as well as in many syndromes including Watson syndrome (pulmonary stenosis/neurofibromatosis), LEOPARD syndrome, Down syndrome, and Williams syndrome. Most of these are relatively easy to differentiate from Alagille syndrome based on other findings. Intrauterine exposure to rubella can lead to cholestasis and pulmonary stenosis as well (usually with other associated anomalies).

Careful discrimination of these aetiologies is necessary because of the extreme differences in prognosis and genetic implications for the family.

Genetics

The familial nature of Alagille syndrome has been recognised from early studies. Watson and Miller[2] studied five families and discussed the possible dominant inheritance and variable expressivity of this disorder. Alagille *et al*[27] found that 3/15 patients had sibs with neonatal cholestasis. Subsequently, additional families were reported with multiple affected members consistent with an autosomal dominant pattern of inheritance with low penetrance and variability of expression.[23 28] Segregation analysis carried out on the families of 33 probands formally corroborated the autosomal dominant inheritance of this disorder and concluded that penetrance is 94%. In this study relatives of probands were considered affected if they showed any one of four features (cardiac abnormality, butterfly vertebra, embryotoxon, or liver disease; facies were excluded because of their subjective nature). Fifteen percent of cases from the entire sample were calculated to be sporadic; however, when only families in which parents were unaffected were considered, 45% were sporadic.[29] This analysis could overestimate the frequency of gene carriers since posterior embryotoxon is found in 8-15% of the general population and heart disease occurs in 8/1000 live births.[30] In another study,[31] the families of 14 patients were studied and six of 24 parents had anomalies in two or more relevant systems, consistent with autosomal dominant inheritance, with 50% of cases representing new mutations. Clearly, while the disease appears to be dominantly inherited, the variable expressivity makes diagnosis of carriers difficult. Without definitive clinical or genetic markers for carriers, counselling for recurrence risks is inaccurate.

Cytogenetics/linkage

Alagille syndrome has been mapped to the short arm of chromosome 20 based on the finding of more than 15 Alagille syndrome patients with a cytogenetically visible deletion or translocation of chromosome 20.[19 25 32-37] Comparison of the cytogenetic breakpoints of the observed abnormalities has led to the assignment of Alagille syndrome to band 20p12 (fig 4). The association of chromosomal deletions with this disorder led to the hypothesis that Alagille syndrome is a contiguous gene deletion syndrome, and may be caused by the deletion of multiple contiguous genes within 20p. However, cytogenetic alterations are found in a small percentage of patients. In a study of 21 patients, one showed a deletion of chromosome 20[36] and in another series, 14 patients were screened and none was found to have a deletion by high resolution techniques.[38] We have studied 56 probands and identified a cytologically balanced translocation in one and a cytogenetically visible deletion in one.[19 37]

A single linkage study carried out in a three generation family showed linkage to two markers within 20p12.[39] A lod score of 2.96 was obtained with a single marker from within 20p12 (D20S27) and when two markers were combined (D20S27 and D20S61) the lod score was 3.56 at zero recombination. Linkage analysis in this disorder is difficult, because variable expressivity makes accurate diagnosis of relatives difficult.

Molecular genetics

Our group has studied 45 patients by molecular analysis of polymorphic markers within 20p12 and identified three deletions (6.7%).[19 40] Two of these patients had cytogenetically visible rearrangements, as discussed above, and the third has normal chromosomes with a submicroscopic deletion. In a study of 23 patients screened by molecular analysis, Deleuze *et al*[41] reported no microdeletions. However, deletions may have been missed in their study, as the five microsatellite markers used span an approximately 30 cM region of 20p12 and were therefore not all closely linked to the Alagille syndome critical region.

The frequency of deletions of 20p in Alagille syndrome (<7%) is significantly lower than that seen in other syndromes hypothesised to be contiguous gene deletion disorders (50-60% in the Prader-Willi syndrome[42-43] and over 80% in the velocardiofacial syndrome[44]). The fact that so few patients with Alagille syndrome have been found to have a deletion argues in favour of there being a single gene causing this disorder. However, it is possible that the distribution of markers within this region is such that we are currently unable to detect small deletions that actually encompass multiple genes.

Comparison of the deletions identified by our group has mapped the critical region to a 1.5 Mb region within 20p12.[19] This region lies within a 3.7 Mb YAC contig.[45] Work towards identification of the disease gene is currently in progress in several laboratories.

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